

**Listing of claims:**

1. **(Withdrawn/Currently amended)** A method for screening substances which are potential inhibitors of ~~transcription~~ expression of bacterial T-box regulated genes, comprising the steps of:

a) incubating one or more assay mixtures to produce a readthrough mRNA product, wherein the assay mixtures comprise ~~comprising~~: a template DNA that comprises: (i) a bacterial promoter, (ii) a glyQS leader ~~of a T-box regulated gene~~, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent ~~magnesium metal~~ cations at a concentration equal to or higher than 30 mM; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the glyQS leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the glyQS leader; and

b) incubating a potential inhibitor substance with one or more assay mixtures to produce a readthrough mRNA product, wherein the assay mixtures comprise ~~comprising~~: a template DNA that comprises: (i) a bacterial promoter, (ii) a glyQS leader ~~of a T-box regulated gene~~, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent ~~magnesium metal~~ cations at a concentration of about 30 mM or higher; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the glyQS leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the glyQS leader; and

c) comparing the ~~amount of fraction of total mRNA products corresponding to~~ the read-through mRNA product produced in step a) with the ~~amount~~ fraction of total mRNA products corresponding to the read-through mRNA product produced in step b)

wherein a lesser ~~amount~~ fraction of ~~a the~~ read-through mRNA product ~~determined for produced in~~ in step b) in comparison with step a) indicates that said potential inhibitor substance inhibits transcriptional readthrough of said ~~T-box~~ glyQS leader and therefore is an inhibitor of expression of bacterial T-box regulated genes.

2. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the divalent metal cation is  $Mg^{2+}$  magnesium concentration is about 30 mM.
3. **(Withdrawn)** The method recited in claim 1 wherein the nucleoside triphosphates are selected from the group consisting of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, and any combination of one or more of these.
4. **(Withdrawn)** The method recited in claim 1 wherein the dinucleotides are selected from the group consisting of ApA, ApC, ApU, ApG, GpA, GpC, GpU, GpG, CpA, CpC, CpU, CpG, UpA, UpC, UpU, and UpG.
5. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the bacterial promoter is selected from the group consisting of ~~the~~ a *B. subtilis* *glyQS* promoter and ~~the~~ a *B. subtilis* *rpsD* promoter.
6. **(Withdrawn)** The method recited in claim 1 wherein the downstream polynucleotide of sufficient length for detection of a read-through mRNA product comprises a polynucleotide which is from about 30 to 150 nucleotide residues in length.
7. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the tRNA specific for a specifier sequence located in the *glyQS* leader is *B. subtilis* tRNA<sup>Gly</sup>.
8. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.
9. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the *glyQS* leader comprises a polynucleotide variant *glyQS* leader sequence which is a variant of a wild-type glycine synthetase *glyQS* leader from a Gram positive bacterial strain, wherein the variant *glyQS* leader sequence comprises modifications to one or both of ~~the wild-type *glyQS* leader~~ specifier and wild-type antiterminator sequences as compared to the wild-type *glyQS* leader.
10. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the tRNA specific for a specifier sequence located in the *glyQS* leader is a variant of a wild-type tRNA ~~wherein any one or more of the~~ in which either or both wild-type anticodon sequence, or the

wild-type discriminator sequence, ~~and the transcription start site is~~ are altered to complement ~~with the~~ the *glyQS* leader sequence.

11. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the *glyQS* leader comprises a variant *glyQS* leader polynucleotide sequence which is a variant of a wild-type glycine synthetase *glyQS* leader sequence from a Gram positive bacterial strain, wherein the variant *glyQS* leader sequence comprises modifications to one or both of the wild-type *glyQS* leader specifier and wild-type antiterminator sequences, and wherein the tRNA specific for a specifier sequence located in the wild-type *glyQS* leader is a variant of a wild-type tRNA wherein ~~in which~~ either or both of the wild-type anticodon sequence, ~~and the~~ or wild-type discriminator sequence are altered to complement ~~with the~~ the variant *glyQS* leader sequence.

12. **(Withdrawn/Currently amended)** The method recited in claim 1 wherein the assay ~~mixtures are~~ comprises an *in vitro* halted-complex bacterial transcription assay systems.

13. **(Withdrawn/Currently amended)** A method for identifying inhibitors of ~~transcription~~ expression of bacterial T-box regulated genes, comprising:

providing two or more *in vitro* halted-complex bacterial transcription assay systems which comprise a template DNA comprising: (i) a bacterial promoter, (ii) a polynucleotide comprising a portion of a leader from ~~the~~ *B. subtilis glyQS* gene and ~~includes~~ including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; RNA polymerase, and uncharged *B. subtilis* tRNA<sup>Gly</sup>, wherein at least one or more of said assay systems comprises a test substance, and wherein at least one or more of said assay systems lacks a test substance, and comparing the ~~amount of~~ fraction of total mRNA products corresponding to *B. subtilis glyQS* read-through mRNA produced in each of said assay systems, wherein a test substance is considered an inhibitor if it effects a lesser ~~amount of~~ fraction of total mRNA products corresponding to the *B. subtilis glyQS* read-through mRNA produced in an assay system comprising ~~such said~~ test substance as compared to an assay system lacking ~~any said~~ test substance.

14. **(Withdrawn/Currently amended)** The method recited in claim 13 wherein the bacterial promoter is selected from the group consisting of the a *B. subtilis* *glyQS* promoter and the a *B. subtilis* *rpsD* promoter.

15. **(Withdrawn/Currently amended)** The method recited in claim 13 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.

16. **(Withdrawn/Currently amended)** The method recited in claim 13 wherein ~~the sequence of the polynucleotide comprising a portion of the leader from the *B. subtilis* *glyQS* leader gene is comprises~~ a variant ~~of the wild-type *B. subtilis* *glyQS* leader sequence, wherein the variant comprises~~ comprising modifications to one or both ~~of the wild-type *B. subtilis* *glyQS* leader specifier and wild-type antiterminator sequences~~ as compared to the wild-type *glyQS* leader, and

wherein the uncharged *B. subtilis* tRNA<sup>Gly</sup> is a variant of a wild-type *B. subtilis* tRNA<sup>Gly</sup> in which either or both wild-type anticodon sequence and wild-type discriminator sequence are altered to complement the variant *B. subtilis* *glyQS* leader sequence.

17. **(Cancelled)**

18. **(Currently amended)** ~~An~~ A purified *in vitro* assay system for screening substances which are potential inhibitors of ~~transcription~~ expression of bacterial T-box regulated genes, comprising:

a) one or more assay mixtures comprising: a template DNA that comprises: (i) a bacterial promoter, (ii) a *glyQS* leader of a T-box regulated gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent ~~magnesium~~ metal cations at a concentration of about 30 mM or higher; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the *glyQS* leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the *glyQS* leader; and

b) one or more assay mixtures comprising: a potential inhibitor substance; a template DNA that comprises: (i) a bacterial promoter, (ii) a *glyQS* leader of a T-box regulated gene,

including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; divalent magnesium metal cations at a concentration of about 3 mM or higher; nucleoside triphosphates; dinucleotides corresponding to and encoded by the transcription start site of the glyQS leader; bacterial RNA polymerase complex; and tRNA specific for a specifier sequence located in the glyQS leader.

19. **(Currently amended)** The assay system recited in claim 18 wherein the divalent ~~metal~~ cation is magnesium cation concentration is about 30 mM.

20. **(Original)** The assay system recited in claim 18 wherein the nucleoside triphosphates are selected from the group consisting of adenosine triphosphate, guanosine triphosphate, cytosine triphosphate, and uridine triphosphate, and any combination of one or more of these.

21. **(Original)** The assay system recited in claim 18 wherein the dinucleotides are selected from the group consisting of ApA, ApC, ApU, ApG, GpA, GpC, GpU, GpG, CpA, CpC, CpU, CpG, UpA, UpC, UpU, and UpG.

22. **(Currently amended)** The assay system recited in claim 18 wherein the bacterial promoter is selected from the group consisting of ~~the a~~ *B. subtilis* glyQS promoter and the a *B. subtilis* rpsD promoter.

23. **(Original)** The assay system recited in claim 18 wherein the downstream polynucleotide of sufficient length for detection of a read-through mRNA product comprises a polynucleotide which is from about 30 to 150 nucleotide residues in length.

24. **(Currently amended)** The assay system recited in claim 18 wherein the tRNA specific for a specifier sequence located in the glyQS leader is *B. subtilis* tRNA<sup>Gly</sup>.

25. **(Currently amended)** The assay system recited in claim 18 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.

26. **(Currently amended)** The assay system recited in claim 18 wherein the glyQS leader comprises a polynucleotide variant glyQS leader sequence which is a variant of a wild-type glycine synthetase glyQS leader from a Gram positive bacterial strain, wherein the variant glyQS

leader sequence comprises modifications to one or both of the wild-type glyQS leader specifier and wild-type antiterminator sequences as compared to the wild-type glyQS leader.

27. **(Currently amended)** The assay system recited in claim 18 wherein the tRNA specific for a specifier located in the glyQS leader is a variant of a wild-type tRNA wherein ~~any one or more of the~~ in which either or both wild-type anticodon sequence, or the wild-type discriminator sequence, and the transcription start site is are altered to complement with the glyQS leader sequence.

28. **(Currently amended)** The assay system recited in claim 18 wherein the leader comprises a polynucleotide variant glyQS leader sequence which is a variant of a wild-type glycine synthetase glyQS leader sequence from a Gram positive bacterial strain, wherein the variant glyQS leader sequence comprises modifications to one or both of the wild-type glyQS leader specifier, and wild-type antiterminator sequences as compared to the wild-type glyQS leader, and wherein the tRNA specific for a specifier sequence located in the ~~wild-type~~ glyQS leader is a variant of a wild-type tRNA wherein in which either or both ~~of the~~ wild-type anticodon sequence, or and the wild-type discriminator sequence are altered to complement with the variant glyQS leader sequence.

29. **(Currently amended)** The assay system recited in claim 18 wherein the assay ~~mixtures~~ are comprises an in vitro halted-complex bacterial transcription assay systems.

30. **(Currently amended)** ~~An~~ A purified in vitro assay system for identifying inhibitors of transcription expression of bacterial T-box regulated genes, comprising:

two or more in vitro halted-complex bacterial transcription assay systems which comprise a template DNA comprising: (i) a bacterial promoter, (ii) a polynucleotide comprising a portion of a leader from the *B. subtilis* glyQS glyQS gene, including a transcription start site, and (iii) a downstream polynucleotide of sufficient length for detection of a read-through mRNA product; RNA polymerase, and uncharged *B. subtilis* subtilis tRNA<sup>Gly</sup>, wherein at least one or more of said assay systems comprises a test substance, and wherein at least one or more of said assay systems lacks a test substance.

31. **(Currently amended)** The assay ~~system~~ recited in claim 30 wherein the bacterial promoter is selected from the group consisting of the a *B. subtilis* *glyQS* promoter and the a *B. subtilis* *rpsD* promoter.

32. **(Currently amended)** The assay ~~system~~ recited in claim 30 wherein the RNA polymerase is purified from either *B. subtilis* or *Escherichia coli*.

33. **(Currently amended)** The assay ~~system~~ recited in claim 30 wherein ~~the sequence of the polynucleotide comprising a portion of the leader from the *B. subtilis* *glyQS* leader gene is comprises a variant of the wild-type *B. subtilis* *glyQS* leader sequence, wherein the variant comprises comprising modifications to one or both of the wild-type *B. subtilis* *glyQS* leader specifier and wild-type antiterminator sequences as compared to the wild-type *glyQS* leader, and wherein the uncharged *B. subtilis* tRNA<sup>Gly</sup> is a variant of a wild-type *B. subtilis* tRNA<sup>Gly</sup> in which either or both wild-type anticodon sequence and wild-type discriminator sequence are altered to complement the variant *B. subtilis* *glyQS* leader sequence.~~

34. **(Cancelled)**

35. **(Cancelled)**